





CERTIFICATION

This is to certify that I have received an accounting from MKULTRA Sub-project # 143 for the period 1 January 1963 - 22 October 1965. The accounting reflects expenses in the amount of \$39,823.29 against the remaining grant balance of \$39,836.00. The residual balance of \$12.71 should be written off as being impracticable for collection or detailed accounting.

The accounting statement and payment certification will be retained by TSD and will be made available for review in TSD if necessary.

I certify that satisfactory services represented by the accounting have been received and that to the best of my knowledge and belief the funds expended were for the purposes authorized by the project approval.

  
Chief, TSD/ES

 

143-9

Page 1 of 2

Statement of Expenditures

Expenditures  
1/1/63-10/22/65

Salaries



\$ 9,089.17  
650.00  
7,710.00  
400.00  
1,995.00  
250.00  
1,458.00  
2,150.00  
2,180.00  
200.00  
75.00  
26,157.17

Total Salaries

Consumable Supplies

F I C A  
Telephone & Telegram  
Tubes & pipettes  
Misc. Supplies  
Electrical Supplies  
Chemicals  
Freight bills  
Filter apparatus  
Cultures  
Flasks, stoppers, syringes, etc.  
Repair on Vacuum pump  
Test tube mixer  
Service on a Sartorius Balance  
Total Consumable Supplies

8.34  
5.55  
575.48  
1,066.35  
115.93  
176.07  
15.33  
113.16  
599.71  
357.99  
89.65  
59.50  
120.00  
3,303.11

Travel



1/31/63-3/6/63,

457.04



4/3/64-4/4/64,

27.90



10/19/64-10/24/64,

21.82



174.25

Continued on next page.

4.1/3



9-13h)

FORM 281: USE PREVIOUS EDITIONS.  
5-80

143-7

143

[REDACTED]

B

77 Dec

E

E

CHECK  
NO.

[REDACTED]

180<sup>00</sup>

12/17

[REDACTED]

A

143

[REDACTED]

B

E

E

CHECK  
NO.

[REDACTED]

AMOUNT OF \$1,500<sup>00</sup>

12/17

[REDACTED]

A

143-8

Date: 8 January 1964

MEMORANDUM FOR : C.TSD/PAES

SUBJECT : MKULTRA, Subproject No. 143

Under the authority granted in the memorandum dated 13 April 1963, from the DCI to the DD/A, and the extension of this authority in subsequent memoranda, Subproject 143 has been approved, and \$20,800.00 of the over-all Project MKULTRA funds have been obligated to cover the subproject's expenses and should be charged to cost center 4125-1390-3902

PROGRAM MANAGER

Concur:

Asst. Chief /s/ 13 JAN 1964  
TSD for D&E

Concur:

APPROVED FOR OBLIGATION  
OF FUNDS:

Chief, DD/P/TSD

Date:

Distribution:

Orig & 1 - Addressee  
2 - TSD/

BB

GROUP I  
Excluded from automatic downgrading and declassification

*Category IV a*



No. 1016

Cost Account 3125-1370-3902

### Object Class

(When Filled In)

143-9  
Date: 11 December 1962

MEMORANDUM FOR: C/TSD/FASS

SUBJECT

MKULTRA, Subproject No. 143

Authorization # 1

Under the authority granted in the memorandum dated 13 April 1953, from the DCI to the DD/A, and the extension of this authority in subsequent memoranda, Subproject 143 has been approved, and \$20,629.44 of the over-all Project MKULTRA funds have been obligated to cover the subproject's expenses and should be charged to cost center 3125-1390-3902

Concur: [REDACTED]

Asst. Chief, TSD for [REDACTED]

Concur: [REDACTED]

APPROVED FOR OBLIGATION  
OF FUNDS: [REDACTED]

I CERTIFY THAT FUNDS ARE AVAILABLE

ORGANIZATION NUMBER 1016 3125-1390-3902  
CHARGE NUMBER [REDACTED]

Date: 11 Dec 1962

Distribution:  
Orig & 1 - Addressee  
2 - TSD/

GROUP 1  
Excluded from auto-  
matic downgrading and  
declassification

**VOUCHER NO. (Finance use only)**

[illegible]

FORM 281 USE PREVIOUS EDITIONS.

(30-40)

REQUEST FOR ADVANCE OF FUNDS	NAME OF ADVANCEE <b>[REDACTED]</b>	DATE 19 December '62	VOUCHER NO. (Finance use only) <b>793-44 (Rev)</b>
PAYABLE TO <b>[REDACTED]</b>	ROOM <b>[REDACTED]</b>	BUILDING <b>[REDACTED]</b>	TELEPHONE EXT. <b>[REDACTED]</b>
<b>\$6 attached Payment Inst.</b>	119		

**Please forward the check to Chief, NSD/Biological Branch through NSD/Sudget Officer by 3 January 1963!**

---

I agree that I will fully account for this advance by submission of vouchers and refund of my unexpended balance to the reporting point stated and by the due date checked below. In the event of my failure to so account and refund my unexpended balance, I authorize deduction from my salary to effect settlement.

DATE	AMOUNT	UNCOMMITTED BALANCE	REPORTING POINT	DUE DATE	V
			FINANCE DIVISION - HEADQUARTERS	065	
<small>ON ARRIVAL AT DESTINATION ON OR ABOUT MONTHLY ON THE LAST WORKDAY OF EACH MONTH</small>					

---

REQUESTING OFFICER: SIGNATURE _____ DATE _____	APPROVED SIGNATURE OF APPROVING OFFICER <b>SUBJECT CERTIFIED AS TRUE</b> DATE _____	RECEIPT FOR FUNDS ADVANCED I received payment of funds in the amount stated hereunder to be used for the purpose stated and accounted for as shown above. SIGNATURE OF ADVANCEE _____ DATE _____ AMOUNT _____
--	--	--

---

I certify funds are available  
CHARGE ALLOTMENT NO. **3125-1390-3002**  
DATE **1016** SIGNATURE \_\_\_\_\_

143-10  
[REDACTED]  
February 10, 1966

[REDACTED]  
Gentlemen:

[REDACTED] notified us that you have requested  
a detailed statement of his grant from [REDACTED].  
We have prepared the detailed statement which you re-  
quire.

Please let us know if there is anything more you need.

Sincerely,  
[REDACTED]

Research Accountant

[REDACTED]  
*accounting to BFRN*  
*23 Jan. 1966*

143-11  
February 23d, 1966

[REDACTED] B  
Attention: [REDACTED] C

Dear [REDACTED]

[REDACTED] told me he discussed the [REDACTED] B  
account with you this morning and it was agreed that:

1. You would again contact the contractor re the \$18,20 unexpended balance reflected in the accounting for the period ending August 31, 1962, and not carried forward to the next grant year;
2. the current grant period would be extended in time only to 30 June 1966, giving the grantee the opportunity to expend in the field the \$123.69 residue, as requested in the January 10th, 1966 letter from [REDACTED] (the original of this letter is enclosed herewith). B

Also enclosed is the original of the February 10th, 1966 letter from the [REDACTED] which forwarded this grantee's accounting for expenditures during the period January 1st, 1963 through October 22nd, 1965.

Thanks very much for your help in the first item.

Enclosure: (2)

#143

143-12

19 January 1966

MEMORANDUM FOR : Chief, TSD/BB

SUBJECT : Delinquent Accountings  
MKULTRA, Subproject 143

1. Review of MKULTRA, Subproject 143 financial records indicate that accountings for grants are now past due. It is requested that your office initiate action to obtain the delinquent accountings.
2. For your information funds have been advanced to the grantee as follows.

Authorization 1 \$19,836.00  
Grant period 1 Jan. - 31 Dec. 1963

Authorization 2 \$20,000.00  
Grant period 1 Jan. - 31 Dec. 1964

Chief, TSD/BB

Distribution:

Original & 1 - Addressee

143-13

E Receipt is hereby acknowledged of [redacted]  
dated 10 February 1984  
drawn on the [redacted] payable to [redacted] - B  
in the amount of  
\$20,000.00.

E Receipt is hereby acknowledged of [redacted]  
dated 7 February 1984  
drawn on the [redacted] payable to [redacted] - B  
in the amount of \$800.00

C [redacted]  
Date Feb. 14, 1964 Secretary



193-19  
MKULTRA

PROJECT CRYPTO : MKULTRA #143

GRANTEE : [REDACTED] B

PRINCIPAL INVESTIGATOR : [REDACTED] C

DATE RENEWED : 1 January 1964

During the past year, the investigators have studied a total of 57 compounds for their capacity to stimulate bacteria. These 57 compounds consisted of 34 phenols, 13 amines, 5 aldehydes and ketones, 3 quaternary ammonium compounds and 2 unclassified chemicals. This study has shown that the phenolic chemicals are the best compounds for stimulating bacteria. Of this group they have noted that Q-phenyl-phenol, p-tert-butyl-phenol and nonyl phenol are the best compounds studied so far.

They have been able to produce as much as a four-fold increase in microbial growth using these compounds. This degree of stimulation is not as great as they had hoped it would be. For this reason, during the past three weeks they changed their line of approach to the problem.

They have produced a product consisting of phenol, casein hydrolyzate, yeast extract and peptone which has a much greater stimulating capacity. It appears that concentrations as small as 50 ppm will give them a consistent 10 fold increase in growth. The deterioration of the cutting fluid is greater in three days than they have ever observed in their studies with these products at any time. In addition, as yet unpublished information from this laboratory indicates that this produce should neutralize practically any known biocide that might be added to cutting fluids.

C It is believed that they have arrived at a point where dynamic progress can be anticipated. In view of the high productivity and promise of results that will be of direct benefit to the operational aspects of DDP. It will be the purpose of the renewed project to combine the two principles of bacterial stimulation (the use of subeffective concentrations of inhibitors and the use of non-specific protective compounds) to determine the ultimate stimulation to be expected in cutting fluids. More emphasis will be given to actual degradative processes rather than stimulation of growth per se. In addition, [REDACTED] has agreed to investigate microbial fouling of diesel cooling systems in conjunction with his other work.

[REDACTED] MKULTRA

(U) Printed on Demand

REQUEST FOR ADVANCE OF FUNDS		NAME OF ADVANCEE A		DATE 30 January 1964		AMOUNT \$600.00 PER VOUCHER NO. (Please use only)	
PAYABLE TO See attached payment incl. E-10		ROOM 30		BUILDING		TELEPHONE EXT.	
PURPOSE: Advance to [redacted] for funding MKULTRA Subproject 143, invoice # 2 which activity was approved by C/TSD on 25 January 1964. Accounting to be in accordance with MKULTRA Fiscal Annex attachment B.							
Please forward checks (2) to C/TSD/33 thru TSD/33 no later than 10 February 1964.							
STATUS OF OUTSTANDING ADVANCES		UNACCOUNTED BALANCE		REPORTING POINT		DUE DATE	
DATE	AMOUNT	FINANCE DIVISION - HEADQUARTERS		ON ARRIVAL AT DESTINATION		MONTHLY " ON THE LAST WORKDAY OF EACH MONTH	
REQUESTING OFFICER		APPROVED		SIGNATURE OF APPROVING OFFICER		RECEIPT FOR FUNDS ADVANCED	
DATE 1/30/64	SIGNATURE [redacted]	OWZ A		SIGNATURE OF APPROVING OFFICER		I acknowledge receipt of funds [redacted] for the purposes stated and accounted for as shown above.	
OBLIGATION REFERENCE NO. 4125-1390-3902		CHARGE ALLOTMENT NO.		CERTIFIED FOR PAYMENT OR CREDIT		DATE	
DATE 4 FEB 1964	SIGNATURE	SIGNED GORTNER, DC/TSD		AUTHORIZED CERTIFYING OFFICER		AMOUNT	
SPACE BELOW FOR EXCLUSIVE USE OF FINANCE DIVISION							
REVIEWED BY							
VOUCHER NO. 7-12							
DESCRIPTION-ALL OTHER ACCOUNTS 13-35		13-35		40-42		47-53	
STATION		STATION		EXPEND. U		OBLIG.	
7-12		7-12		PER. NO.		NO.	
P.O. NO.		P.O. NO.		L.I.O. NO.		L.I.O. NO.	
PROJECT NO.		PROJECT NO.		CODE		CODE	
ADVANCE ACCOUNTS 13-27		ADVANCE ACCOUNTS 13-27		GENERAL		GENERAL	
7-12		7-12		V.A.		V.A.	
7-12		7-12		ACCT. NO.		ACCT. NO.	
7-12		7-12		EMP. NO.		EMP. NO.	
7-12		7-12		56-67		56-67	
7-12		7-12		ALLOT. OR COST		ALLOT. OR COST	
7-12		7-12		ACCT. NO.		ACCT. NO.	
7-12		7-12		54-57		54-57	
7-12		7-12		GENERAL		GENERAL	
7-12		7-12		V.A.		V.A.	
7-12		7-12		ACCT. NO.		ACCT. NO.	
7-12		7-12		EMP. NO.		EMP. NO.	
7-12		7-12		56-67		56-67	
7-12		7-12		ALLOT. OR COST		ALLOT. OR COST	
7-12		7-12		ACCT. NO.		ACCT. NO.	
7-12		7-12		54-57		54-57	
7-12		7-12		GENERAL		GENERAL	
7-12		7-12		V.A.		V.A.	
7-12		7-12		ACCT. NO.		ACCT. NO.	
7-12		7-12		EMP. NO.		EMP. NO.	
7-12		7-12		56-67		56-67	
7-12		7-12		ALLOT. OR COST		ALLOT. OR COST	
7-12		7-12		ACCT. NO.		ACCT. NO.	
7-12		7-12		54-57		54-57	
7-12		7-12		GENERAL		GENERAL	
7-12		7-12		V.A.		V.A.	
7-12		7-12		ACCT. NO.		ACCT. NO.	
7-12		7-12		EMP. NO.		EMP. NO.	
7-12		7-12		56-67		56-67	
7-12		7-12		ALLOT. OR COST		ALLOT. OR COST	
7-12		7-12		ACCT. NO.		ACCT. NO.	
7-12		7-12		54-57		54-57	
7-12		7-12		GENERAL		GENERAL	
7-12		7-12		V.A.		V.A.	
7-12		7-12		ACCT. NO.		ACCT. NO.	
7-12		7-12		EMP. NO.		EMP. NO.	
7-12		7-12		56-67		56-67	
7-12		7-12		ALLOT. OR COST		ALLOT. OR COST	
7-12		7-12		ACCT. NO.		ACCT. NO.	
7-12		7-12		54-57		54-57	
7-12		7-12		GENERAL		GENERAL	
7-12		7-12		V.A.		V.A.	
7-12		7-12		ACCT. NO.		ACCT. NO.	
7-12		7-12		EMP. NO.		EMP. NO.	
7-12		7-12		56-67		56-67	
7-12		7-12		ALLOT. OR COST		ALLOT. OR COST	
7-12		7-12		ACCT. NO.		ACCT. NO.	
7-12		7-12		54-57		54-57	
7-12		7-12		GENERAL		GENERAL	
7-12		7-12		V.A.		V.A.	
7-12		7-12		ACCT. NO.		ACCT. NO.	

743-18  
Date 8 January 1964

**COVERT ACTION SUPPORT**

Branch BB Category Sabotage ( IVa )

Project Title \_\_\_\_\_ Item Classification Unclassified (Data)

Project Crypto. MKULTRA 143 Crypto Classification Unclassified

Branch Project No. N.A. Project Engineer. [REDACTED] **A**

Contractor [REDACTED] **C**

Contract No. N.A. Task No. N.A.

Type of Contract MKULTRA Date Initiated 1 January 1964

**FY 63 - \$20,629.44**  
Cost **FY 64 - \$20,800.00** Completion Date 1 year

Purpose: The objective of the first year was achieved. An approach to the stimulation of soluble-oil degradation was proved feasible. In addition a system for negating microbial inhibitors was developed. These two accomplishments appear to be the first significant breakthrough for microbial degradation of materials. It is planned to study further the limitations of these approaches and their application to a variety of practical situations during the coming year.

Status: Performance has been excellent. Has progressed beyond expectations. Current in fiscal matters.

Requirement: Generated by undocumented requests from DDP units and divisions as well as necessity for TSD to maintain a quick delivery capability to meet anticipated future operational needs.

[REDACTED]

4. The cost of this program for the second year is \$20,000.00. To this sum must be added \$800.00 representing a four per cent service charge to the [REDACTED] } B

The [REDACTED] will function as cut-out and cover for this grant. The cover title of the project is [REDACTED] -C

5. Total cost of the project will not exceed \$20,800.00. Charges should be made against Allotment No. 4125-1390-3902.

6. The [REDACTED] will request the [REDACTED] to submit a summary accounting of monies received and to return any unexpended grant funds at the end of the project year. B

7. Title to any permanent equipment purchased by funds granted the [REDACTED] shall be retained by them in lieu of higher overhead rates. B

8. It was mutually agreed that documentation and accounting for travel expenses which are reimbursable by the [REDACTED] shall conform with the accepted practices of that institution. B  
A

[REDACTED]  
TSD/Biological Branch

Attachments:  
Proposal & budget

Note: Due date for accounting of previous year's funds is 31 March 1964.

Distribution:  
Original only

143-17  
MKULTRA

DRAFT

8 January 1964

MEMORANDUM FOR : THE RECORD

SUBJECT : MKULTRA, Subproject 143

1. The purpose of subproject 143 is to continue support of the work of [REDACTED] at the [REDACTED] on an approach to microbial deterioration of materials. 3B

2. During the first year a system was developed which allows a 10 fold stimulation of the microbial deterioration of soluble-oil coolants (as a type system) using extremely small quantities of active material. In addition a system was found which apparently serves to inactivate most microbial inhibitors in such a product. This was much more of an accomplishment than was expected. For the coming year it is planned to explore the limitations of these approaches as they apply to cutting oils and other petroleum based products. It is hoped that these techniques will be applicable across-the-board to all sabotage or harassment systems involving microorganisms, promoting a more deliverable package to the operator.

3. [REDACTED] principal investigator, has been cleared through SECRET. C

[REDACTED] MKULTRA

143-78

DATE: 8 January 1964

MEMORANDUM FOR : C TSD FASS

SUBJECT : MKULTRA, Subproject No. 143

Under the authority granted in the memorandum dated 18 April 1953 from the DCI to the DD/A, and the extension of this authority in subsequent memoranda, Subproject 143 has been approved, and \$20,800.00 of the over-all Project MKULTRA funds have been obligated to cover the subproject's expenses and should be charged to cost center 4125-1390-3902

A

[REDACTED]

Concur:

[REDACTED]

100-441111-100000  
CHARGE TO ACCOUNT NO. 4125-1390-3901

AUTHORIZING OFFICER

APPROVED FOR OBLIGATION  
OF FUNDS

[REDACTED]

Date:

Distribution:

Orig & 1 - Addressee  
2 - TSD, BB

GROUP I

Excluded from automatic downgrading and declassification

143-19

January 3, 1964

Dear [REDACTED]

As you know, the grant from the [REDACTED] is one year old and it is time to consider renewal of the grant. B

During the past year we have studied a total of 57 compounds for their capacity to stimulate bacteria. These 57 compounds consisted of 34 phenols, 13 amines, 5 aldehydes and ketones, 3 quaternary ammonium compounds and 2 unclassified chemicals. This study has shown that the phenolic chemicals are the best compounds for stimulating bacteria. Of this group we have noted that Q-phenyl-phenol, p-test-butylphenol, di-sec-butylphenol, dodecyl phenol, 2,4-dimethyl-6-test-butylphenol and nonyl phenol are the best compounds studied so far.

We have been able to produce as much as a four-fold increase in microbial growth using these compounds. This degree of stimulation is not as great as we had hoped it would be. For this reason, during the past three weeks we changed our line of approach to the problem.

We have produced a product consisting of phenol, casein, hydrolyzate, yeast extract and peptone which has a much greater stimulating capacity. It appears that concentrations as small as 50 ppm will give us a consistent 10 fold increase in growth. The deterioration of the cutting fluid is greater in three days than we have ever observed in our studies with these products at any time. In addition, as yet unpublished information from this laboratory indicates that this product should neutralize practically any known biocide or stat that might be added to cutting fluids.

We feel that we now have arrived at a point where we can make some dynamic progress. During the coming year we hope to:

1. Study additional chemicals and media products for their stimulatory capacity.
2. Determine the best ratios of the media components and generally improve the formulation.
3. Determine the effect of the formulation on other petroleum products.
4. Determine if the formulation has the capacity to neutralize anti-microbial agents.
5. Begin work on how the formulation stimulates microorganisms.
6. Determine the effect of the formulation plus urine on microbial growth.

I believe these points cover the main objectives for the coming year.

Kindest personal regards.

Sincerely yours,

C

143-19

PROPOSED GRANT COST

A. Principal Investigator ( $\frac{1}{4}$ time)	3990.00
Full time research assistants	9417.00
B. Expendable equipment and supplies	2560.00
C. Typing reports, papers, etc.	700.00
D. Overhead (20%)	3333.00
TOTAL	20,000.00



143-20

27 January 1964

MEMORANDUM FOR THE RECORD

SUBJECT: MKULTRA Subproject 143

1. At the request of both Chief and Deputy Chief, TSD [REDACTED] was contacted for the purpose of funding this MKULTRA Subproject through one of the established funding mechanisms controlled by [REDACTED]. Due primarily to the funding date, it was not possible to accomplish this at this time.
2. Arrangements will be made with [REDACTED] to project our funding requirements for MKULTRA Subprojects on a semi-annual basis. With this lead time, [REDACTED] feels that they may be capable of funding our total requirements.
3. [REDACTED] will meet with [REDACTED] to discuss other Subprojects funding mechanisms at 1115 this date.

G

A

[REDACTED]  
Chief, TSD/Support Staff

GROUP 1  
Excluded from automatic  
downgrading and  
declassification

193-21  
REVISED

Date 3 September 1963

COVERT ACTION SUPPORT

Branch BB Category Sabotage (formerly IIIb) (IVa)

Project Title \_\_\_\_\_ Item Classification Unclassified (Data)

Project Crypto MKULTRA 143 Crypto Classification Unclassified

Branch Project No N.A. Project Engineer [REDACTED] A

Contractor [REDACTED] C

Contract No N.A. Task No N.A.

Type of Contract MKULTRA Date Initiated 1 January

Cost \$20,629.44 Completion Date 1 year

Purpose: To investigate the potential of a new approach to the stimulation of microbial deterioration of petroleum products and its possible application to other degredative processes.

Status: Contractor has developed a model system for desired system. Will now attempt to apply it on small scale. Current in fiscal matters.

Requirement: Generated by undocumented requests from DDP units and divisions as well as necessity for TSD to maintain a quick delivery capability to meet anticipated future operational needs.

143-22

RECEIPT

Receipt is hereby acknowledged of [REDACTED]  
dated January 7, 1963, drawn on [REDACTED]  
payable to [REDACTED]  
in the amount of \$19,836.00

E

B

Receipt is hereby acknowledged of [REDACTED]  
dated January 7, 1963, drawn on [REDACTED]  
payable to [REDACTED]  
in the amount of \$175.44

E

B

C

Date

Jan 10 1963

143-23

DRAFT

11 December 1962

MEMO FOR : THE RECORD

SUBJECT : MAULTRA, Subproject 143

1. The purpose of subproject 143 is to support the work of [REDACTED] C & B  
[REDACTED] on an approach to the stimulation of petroleum product deterioration.
2. Although the model system for these studies will be petroleum products, this approach to stimulation of deterioration may have wide application to all areas of biological sabotage currently of interest to TSD.
3. [REDACTED] principal investigator, has been cleared through SECRET. C
4. The cost of this program for the first year is \$19,836.00. To this sum must be added \$793.44 representing a four per cent service charge to the [REDACTED] will B  
function as cut out and cover for this grant. The cover title of the project is [REDACTED] } C  
[REDACTED]
5. Total cost of the project for a period of one year will not exceed \$20,629.44. Charges should be made against Allotment No.

143-23

- 2 -

3125-1390-3902.

6 [REDACTED] Will request the [REDACTED] to submit a summary accounting of monies received and to return any unexpended grant funds at the end of the project year.

7. Title to any permanent equipment purchased by funds granted the [REDACTED] shall be retained by them in lieu of higher overhead rates.

8. It was mutually agreed that documentation and accounting for travel expenses which are reimbursable by the [REDACTED] shall conform with the accepted practices of that institution.

9. The requirement for a six months' informal accounting on the part of the principal investigator is waived.

[REDACTED]  
TSD/Biological Branch

[REDACTED]  
TSD/Biological Branch

Attachment:  
Proposal & Budget

Distribution:  
Original only

[REDACTED]

148-43

GRANT REQUEST

from



C+B

---

Biographical Sketch

Entire page

C

Publications and Papers:

5 pages

C

Symposia and Books

1 page



743-25  
B  
FACILITIES

[REDACTED] and can place the following equipment at the disposal of this project:

Balances (Sartorius, etc.)

Warburg

Ultra-centrifuge

Ratheon Sonic Oscillator

Mickle Disintegrator

Centrifuges (refrigerated, Sorval, International, Ultra-centrifuge, Spinco, etc.)

Sharples Super Centrifuge

Beckman DU 60 Spectrophotometers

Beckman B

Coleman Photofluorometer

Refrigerated Warburg

Incubators

Research pH Meters

Shaking Incubators

Kjeldahl Apparatus

Colorimeters

Pilot Plant Fermentors

Chromatography Equipment

Fraction collectors

Isotope laboratory with complete supporting equipment

14-23

## OBJECTIVE OF THIS INVESTIGATION

To determine if the growth of microorganisms in petroleum products can be markedly increased by the addition of substatic concentrations of antimicrobial agents.

## LITERATURE REVIEW

The literature has been carefully searched to determine what is known concerning the use of antimicrobial agents to stimulate microbial growth. To date, only two papers have been found that relate to this topic.

Imshenetskii and Perova (Institute of Microbiology, Academy of Science U.S.S.R., Mikrobiologiya, 23, 53-56, 1954) found that cultures of Saccharomyces cerevisiae trained to grow in the presence of small concentrations of  $HgCl_2$  or phenols have much higher fermentation rates than cultures that have not been trained to grow in the presence of these compounds. As little as 50 ppm of  $HgCl_2$  accelerates the production of beer. These workers believe that this effect is due to traces of inhibitor in the environment and not because of some change in the physiology of the cells.

C

[REDACTED]

reported that 50 ppm of 2,4,6-trichloro phenol could markedly stimulate the growth of bacteria in emulsion oils. In some instances this compound produced almost a three fold increase in the number of cells in the environment.

C

[REDACTED]

found that 25 to 150 ppm of 2,4-dinitrophenol increases the oxidation of  $C_4$ ,  $C_6$ ,  $C_8$ ,  $C_{10}$ ,  $C_{12}$ ,  $C_{13}$ ,  $C_{14}$ , and 66 fatty acids. The increase was as much as 100% and was not associated with an increase in

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the number of cells in the environment.

These results imply that two types of stimulation may be produced by substatic concentrations of inhibitors. First, a metabolic stimulation may be produced where the cells do not increase in number but this metabolic rate is increased. Second, the number of cells in the environment may be increased by the compound. It remains to be seen whether there is a stimulation in the individual metabolic activity of each cell in this case. There is an increase in activity, however, this may only be due to the greater number of cells.

#### UNPUBLISHED DATA

\_\_\_\_\_ have found that 1000 ppm of 2-nitro-1-butyl acetate produce a 3 fold increase in the production of penicillin by Penicillium chrysogenum.

#### PLAN OF ATTACK

It is well known that the rate of oxidation of hydrocarbons may be limited by the rate of diffusion of the compound across the permeability barrier. It is also well known that surface active agents increase the permeability of the cytoplasmic membrane. Several years ago \_\_\_\_\_ developed the idea that the use of surface active compounds may increase the accessibility of hydrocarbon substrates to microbial attack and thus speed up the oxidation of these compounds.

Due to several factors including a lack of funds, this idea has never been tested to any extent. To date, we have studied 3 phenolic compounds. Two of these compounds produced stimulation and one did not.

We propose to study the effect of substatic concentrations of  
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phenols, fatty amines/alcohols and quaternary ammonium compounds on the oxidation of two different synthetic coolants and one petroleum type product. We may also use other antimicrobial agents other than those listed above.

## EXPERIMENTAL PROCEDURE

### CULTURES

Pure cultures of microorganisms belonging to the Genus Pseudomonas will be used in this investigation. The cultures will be isolated from samples of used cutting fluids obtained in the Houston area.

### MEDIA

Whenever possible, the organisms will be maintained in cutting fluids. In some instances conventional media such as nutrient broth, meat infusion broth, or an inorganic salts medium may be used in this investigation.

### INCUBATION TEMPERATURE

All experiments will be done at 30°C. The length of the incubation period will vary according to the organism and the particular experiment.

### CHEMICALS

The best grade of chemical obtainable will be used throughout this investigation.

### GLASSWARE

All flasks, tubes and other glass equipment will be acid cleaned prior to each experiment.

### HYDROGEN ION CONCENTRATION

The pH of the environment will vary according to the particular experiment. In general, we will let the Ph of the cutting fluid

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determine the pH of the experiment.

#### INOCULUM

The cells used in the experiments will be grown on an appropriate medium. They will be harvested after a suitable incubation period and washed three times in isotonic saline. The cells will be suspended in isotonic saline and uniform quantities of this suspension will be used to inoculate the tubes or flasks.

#### STERILITY

We will employ suitable procedures for sterilizing the media containing the petroleum products.

#### CONTROLS

Appropriate controls will be included in each experiment. Each experiment will be done in duplicate and repeated at least once.

#### CUTTING FLUIDS

Three different cutting fluids will be used in this investigation consisting of two chemical coolants and one petroleum product.

#### EXPERIMENTAL TECHNIQUE

Each cutting fluid will be prepared in a 1:25 dilution in tap water. One hundred ml of this coolant will be placed in each 250 ml Erlenmeyer flask. The number of flasks used in each experiment will vary from time to time. The inhibitor will be added to the series of flasks in varying concentrations. These flasks will be sterilized by autoclaving and then inoculated with the test organisms. The flasks will be incubated for a period of time (probably two weeks) in a shaking incubator.

At periodic intervals of about 2 days time the number of cells per ml will be determined for each flask. At the same time the pH of the flask will be determined and other biochemical tests (such as dehydrogenase activity) may be conducted to determine the degree of metabolic activity in the environment. At the end of the experiment

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the remaining cutting fluid will be poured into a volumetric cylinder to determine the quantity of free oil in the product (this test will be used on the petroleum coolant only). The quantity of free oil present is an excellent index of the degree of attack on the emulsifier.

In addition, Warburg studies will be conducted at the optimum stimulatory concentration determined in the growth expedient to establish the effect of the antimicrobial agent on oxygen uptake.

We will study as many compounds as possible during the year period of this grant. In no case will this be less than twenty-five compounds.

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COST OF PROJECT

A. Principal Investigator \$200.00/month (9 months) \$ 1,800.00

Full time salary to work on project in  
Summer 3,210.00

2 Full Time Research Assistants (\$375/month each) 9,000.00

\$ 14,010.00

B. Permanent Equipment

1 Shaking incubator \$ 1,080.00

C. Expendable Equipment & Supplies \$ 900.00

D. Publication Cost

Typing report, papers, and purchasing reprints  
of publications that may arise from this work \$ 400.00

\$ 16,390.00

E. Overhead (20%)

\$ 3,446.00

\$ 19,836.00